BIOLOGY OF SPONGE NATURAL PRODUCTS. *Memoirs of the Queensland Museum 44: 590. 1999*:- This is to announce the EC-MAS 3 project, which started April 1, 1998. The biological and chemical aspects of selected sponge natural products (secondary metabolites) of interest to human use will be studied to obtain understanding of: 1) the cellular origin and possible microsymbiont involvement, and 2) the ecological significance of sponge secondary metabolites, and 3) the patterns in these processes enabling rationalisation of exploration for and exploitation of sponge secondary metabolites. The results will have a direct bearing on policy decisions concerning industrial production of sponge secondary metabolites, which are too difficult or too costly to synthesise. A major deliverable product of the proposed research will be the formulation of a standard protocol of research steps needed as a basis for such policy decisions.

The research will be structured in three phases: 1) exploration and pattern recognition, 2) testing of hypotheses using experiments with selected sponges, 3) protocol construction. Initially, investigations will be directed towards two sponge groups (Haplosclerida and Halichondrida) and towards a limited number of molecule types. Known secondary metabolite occurrence will direct exploration for related sponges and related secondary metabolites. For the experimental phase a choice for 3-4 target sponges will be made based on suspected production of secondary metabolites by own sponge cells (1-2 target sponges) or microsymbionts (1-2 target sponges). The biological aspects include: determination of the identities and phylogenetic relationships of bioactive sponges; within-sponge spatial distribution of sponge cells and microsymbiont cells; experimental observation of variability of biological activity of selected sponges in various environmental (biotic and abiotic) situations; identification of target microsymbiont cells; fractionation, isolation and culture of target sponge cells. The chemical aspects include: extraction, isolation and structure determination of selected bioactive compounds; development of qualitative and quantitative analytical methods for their spatial distribution in the sponge and for their distribution between and within different species.

Summary of methodologies: Sponges will be collected using SCUBA (shallow water) and/or dredges (deep water) and photographed upon collection. Various types of fixations of material will be made immediately after removal from the water. Voucher specimens will be studied for identity and phylogeny using routine morphological as well as molecular (18S / 28S rDNA) characters. Collected sponges preserved in methanol or as freeze-dried material will be extracted with methanol and dichloromethane. The primary extracts will then be tested for their biotoxicity using an invertebrate bioassay organism, the Artemia toxicity test, and several prokaryote and eukaryote bioassay organisms (bacteria, fungi, yeast). Cytological analyses will consist of two different approaches, one using glutaraldehyde-fixed material, the other using live sponges: 1) sponges will be fixed in glutaraldehyde, (a) for microsymbiont detection; thick sections will be stained with suitable fluorochromes and viewed by fluorescence microscopy and confocal scanning light microscopy. If microsymbionts are present, populations will be characterised by different parameters. Microsymbionts will be further identified by fluorescence in situ hybridisation using rRNA-targeted oligonucleotides as probes; (b) for sponge cell spatial distribution, samples will be postfixed in 1% osmium tetroxide and thin sections will be studied by Transmission Electron Microscopy (TEM). 2) Live sponges will be dissociated into single-cell suspensions. Recognition of secondary metabolite production will be realised using two advanced techniques: (a) cell fractionation into pure cell populations using continuous or discontinuous Percoll gradients; (b) symbiont-free sponge cultures, initiated either from pure cell populations or from dissociated sponge cell suspensions. Experimental observations will be made in situ using various types of manipulations (caging, artificial standard lesions, confrontation with substrate competitors, crude extract assays with substrate competitors and potential predators). ❐ *Porifera, secondary metabolites, microsymbionts, chemical ecology, exploration and exploitation.*

R.W.M. van Soest (email: soest@bio.uva.nl), Department of Coelenterates and Porifera, Institute for Systematics and Population Biology (Zoologisch Museum), University of Amsterdam, P.O.Box 94766, 1090 AT Amsterdam, The Netherlands; G. Van De Vyver & E. Richelle-Maurer, Physiologie Cellulaire et Genetique des Levures, Université Libre de Bruxelles, Bd du Triomphe - CP 244, B-1050 Bruxelles, Belgium; C. Woldringh, J.C. Braekman & R. Tavares, Université Libre de Bruxelles, Faculté des Sciences, Laboratoire de Chimie Bio-Organique CP 160/70, Avenue F.D. Roosevelt 50, B-1050 Bruxelles, Belgium; 1 June 1998.